Clinical Infectious Diseases
MAJOR ARTICLE

IDSA Infectious Diseases Society of America hivma hiv medicine association OXFORD

Effects of Immunonutrition in Advanced Human Immunodeficiency Virus Disease: A Randomized Placebo-controlled Clinical Trial (Promaltia Study)

Sergio Serrano-Villar,[1] María de Lagarde,[2] Jorge Vázquez-Castellanos,[3] Alejandro Vallejo,[1] José I. Bernadino,[4] Nadia Madrid,[1] Mariano Matarranz,[2] Alberto Díaz-Santiago,[1] Carolina Gutiérrez,[1] Alfonso Cabello,[5] Judit Villar-García,[6] José Ramón Blanco,[7] Otilia Bisbal,[2] Talía Sainz,[8] Andrés Moya,[3,9,10] Santiago Moreno,[2] María José Gosalbes,[3,10] and Vicente Estrada[11]

[1]Department of Infectious Diseases, Hospital Universitario Ramon y Cajal, Facultad de Medicina, Universidad de Alcalá, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), and [2]HIV Unit, Hospital Universitario Doce de Octubre, Madrid, [3]Area of Genomics and Health, FISABIO-Salud Pública, Valencia, [4]HIV Unit, Hospital Universitario La Paz, Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), and [5]Infectious Diseases Division, Hospital Universitario Fundación Jiménez Díaz, Madrid, [6]Infectious Diseases Department, Hospital del Mar, Instituto Hospital del Mar de Investigaciones Médicas (IMIM), Barcelona, [7]Department of Infectious Diseases, Hospital San Pedro – Centro de Investigación Biomédica de la Rioja (CIBIR), Logroño, [8]Pediatric Tropical and Infectious Diseases Department, Hospital Universitario La Paz, Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), [9]Institute of Integrative Systems Biology, University of Valencia and CSIC, Valencia, [10]CIBER in Epidemiology and Public Health, Madrid, and [11]HIV Unit, Hospital Clínico San Carlos, Madrid, Spain

Background. While nutritional interventions with prebiotics and probiotics seem to exert immunological effects, their clinical implications in human immunodeficiency virus (HIV)–infected subjects initiating antiretroviral therapy (ART) at advanced HIV disease remain unclear.

Methods. This was a pilot multicenter randomized, placebo-controlled, double-blind study in which 78 HIV-infected, ART-naive subjects with <350 CD4 T cells/μL or AIDS were randomized to either daily PMT25341 (a mixture of synbiotics, omega-3/6 fatty acids and amino acids) or placebo for 48 weeks, each in combination with first-line ART. Primary endpoints were changes in CD4 T-cell counts and CD4/CD8 ratio from baseline to week 48 and safety. Secondary endpoints were changes in markers of T-cell activation, bacterial translocation, inflammation, and α and β microbiota diversity.

Results. Fifty-nine participants completed the follow-up with a mean $CD4^+$ T-cell count of 221 ± 108 cells/μL and mean CD4/CD8 ratio of 0.26 ± 0.19. PMT25341 was well tolerated, without grade 3–4 adverse effects attributable to the intervention. While most of the assessed biomarkers improved during the follow-up in both arms, PMT25341-treated subjects did not experience any significant change, compared to placebo-treated subjects, in mean $CD4^+$ T-cell count change (278 vs 250 cells/μL, $P = .474$) or CD4/CD8 ratio change (0.30 vs 0.32, $P = .854$). Similarly, we did not detect differences between treatment arms in secondary endpoints.

Conclusions. In HIV-infected patients initiating ART at advanced disease, the clear immunological benefits of ART were not enhanced by this nutritional intervention targeting the gut-associated lymphoid tissue and microbiota.

Clinical Trials Registration. NCT00870363.

Keywords. microbiota; CD4 T cells; probiotics; immunoactivation; inflammation.

Despite global efforts to identify human immunodeficiency virus (HIV) infection at an early stage, HIV is diagnosed late in approximately 50% of cases in high-income countries [1, 2]. According to the consensus definition from the HIV in Europe Study Group, late presenters are defined as those presenting to an antiretroviral therapy (ART) clinic with a CD4 count of <350 cells/μL or an AIDS-defining illness [3]. Low $CD4^+$ T-cell counts at ART initiation have been associated with shorter life expectancy [4]. Hence, while early diagnosis and ART initiation must be prioritized in public health strategies, novel interventions are required for a more successful management of HIV in late-presenter cases.

An increased level of inflammation, immune activation, and low $CD4^+$ T-cell nadir is seen in late presenters, along with an impaired immunological recovery during ART [5]. Starting early in HIV infection, the gut-associated lymphoid tissue (GALT) serves as a sanctuary for HIV replication and a portal of systemic inflammation, likely contributing to residual morbidity [6]. A greater decline of circulating $CD4^+$ T cells also limits ART-mediated GALT restoration by impairing the number of T cells trafficking to the gut [7, 8], allowing for compositional [9, 10] and functional [11] changes in the microbiota. Indeed, low $CD4^+$ T-cell counts in peripheral blood are associated with impaired epithelial proliferation, increased neutrophil infiltration, and mucosal apoptosis in colorectal biopsies, which correlate with the independent predictors of mortality [12, 13].

Different strategies targeting the multifaceted HIV-associated GALT defects have been investigated in an attempt to reduce

Received 6 March 2018; editorial decision 3 May 2018; accepted 9 May 2018; published online May 16, 2018.

Correspondence: S. Serrano-Villar, Department of Infectious Diseases, Hospital Universitario Ramon y Cajal, Facultad de Medicina, Universidad de Alcalá (IRYCIS), Carretera de Colmenar Viejo, Km 9.100, Madrid 28034, Spain (sergio.serrano@salud.madrid.org).

Clinical Infectious Diseases® 2019;68(1):120–30

© The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.

DOI: 10.1093/cid/ciy414

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

the long-term consequences of chronic inflammation [14–19]. Multiple studies have assessed dietary supplementation in HIV patients with various nutritional products, such as prebiotics and probiotics, among others [10, 14–16, 20], which collectively suggest that this strategy may exert some beneficial immunological effects. Therefore, we developed PMT25341, a product including several nutritional compounds designed to target the different gastrointestinal defects associated with HIV immunopathogenesis. We conducted a double-blind, randomized, placebo-controlled study to evaluate whether PMT25341 administered for 48 weeks in late presenters initiating ART may enhance CD4$^+$ T-cell and CD4/CD8 ratio recovery and affect markers of immune activation, inflammation, and bacterial translocation, as well as the gut microbiota composition.

METHODS

Study Design, Participants, Setting, and Eligibility

We conducted a randomized, double-blind, multicenter, placebo-controlled study. Participants were recruited at the HIV units of 7 university hospitals in Spain—Hospital Universitario Ramón y Cajal, Hospital Universitario Clínico San Carlos, Hospital Universitario Doce de Octubre, Hospital Universitario La Paz, Fundación Jiménez Díaz (Madrid), Hospital del Mar (Barcelona), and Hospital San Pedro (Logroño). Participants were HIV-infected, late-presenting (<350 CD4 T cells/µL or AIDS at diagnosis [3]) adults, who initiated ART according to the Spanish Grupo de estudio del SIDA (GESIDA) National Guidelines [21]. Exclusion criteria were age <18 years, pregnancy, type 1 or 2 diabetes, end-stage renal disease, lactose intolerance, use of immunomodulatory drugs, and a neutrophil count of <750 cells/µL. The composition of PMT25341 is detailed in Supplementary Table 1; the placebo was skimmed milk powder. The product and the placebo were prepared by Nutricion Médica, S.L.

The study was approved by the ethics committee of all participating centers and all participants signed an informed consent prior to the initiation of study procedures. This study was registered at ClinicalTrials.gov (identifier NCT03009032).

Randomization

The study participants were randomly assigned to PMT25341 or placebo by a computer-generated randomized number system in blocks. PMT25341 and the placebo were packaged in identically appearing sachets. The clinicians who saw the study participants, the laboratory personnel who handled patient specimens, and the study participants were blinded to the assigned patient group.

Study Outcomes

After screening for eligibility, study visits were scheduled at baseline, and at 4, 24, and 48 weeks. At each visit, a clinical evaluation was carried out, blood samples were collected, and adherence to the intervention and any adverse events were registered. Fecal samples were collected at baseline and week 48. Our primary outcomes were safety and tolerability, and between-arm changes in CD4$^+$ and CD4/CD8 ratio from baseline to week 48. Gastrointestinal (GI) tolerance was also assessed using a questionnaire and scoring the severity of GI symptoms on a 4-point scale.

Our secondary outcomes were changes in plasma markers (interleukin 6 [IL-6], high-sensitivity C-reactive protein [hs-CRP], tumor necrosis factor alpha [TNF-α], soluble CD163 [sCD163], soluble CD14 [sCD14], interferon-gamma-inducible protein 10 [IP-10], lipoteichoic acid [LTA], interleukin 7 [IL-7], interleukin 10 [IL-10], and interleukin 17 [IL-17]), T-cell activation markers (percentage of HLA-DR$^+$CD38$^+$ CD4$^+$ and CD8$^+$ T cells), T-cell senescence markers (percentage of CD28$^-$ T cells), and variations in the microbiota composition (α- and β-diversity metrics) from baseline to week 48. Adherence was assessed using patient diaries that were checked against the returned sachets.

Nucleic Acid Purification, Amplification of the 16S Ribosomal RNA Gene, Sequencing, and Bioinformatics Analysis

Nucleic Acid Purification

Fecal samples were stored in RNAlater (Life Technologies) at −80°C until use. Total DNA was quantified using Qubit fluorometry.

Amplification of the 16S Ribosomal RNA Gene

For each sample, the V3–V4 regions of the 16S ribosomal RNA (rRNA) gene were amplified by polymerase chain reaction (PCR) using the 16S amplicon PCR forward primer (5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) wand 16S Amplicon PCR Reverse Primer (5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC). The pooled PCR products were sequenced using the kit V3 (2 × 300 cycles) with MiSeq sequencer (Illumina) at FISABIO Sequencing and Bioinformatics Service, Valencia, Spain. We obtained an average of 62 939 16S rRNA joined sequences per sample.

16S RNA Gene Analysis. Biodiversity, and Clustering

Amplicon data from the 16S rRNA gene was analyzed following the recommendations of the metagenomic state-of-the-art pipeline QIIME version 1.9 [22]. Taxonomic information on the 16S rDNA sequences were obtained using the Ribosomal Database Project II [23] and the Greengenes database available in QIIME version 1.8 software. Operational taxonomic units (OTUs) were created using the QIIME version 1.9 script pick_open_reference_otus.py, which iteratively uses Uclust [24], and were used to classify different clusters of species with a 97% similarity. OTUs were created using Uclust [25], applying the 97% similarity criterion. Representative sequences were aligned with Pynast [24] against the clustered version of the Greengenes database (database core_set_aligned.fasta.imputed), to use as input to reconstruct the phylogenetic tree using the FastTree software [26]. Distance analysis between samples was performed by the weighted normalized

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

UniFrac using the script assign_taxonomybeta_diversity.py. Sequences were rarefacted to 16 590 sequences per sample to standardize α- and β-diversity analyses. A detailed description of the methods is provided in the Supplementary Materials.

Systemic Biomarkers of Disease Progression

Markers of Innate Immune Activation and Bacterial Translocation

A sample of fasting venous blood was obtained to determine the concentrations of glucose, total cholesterol, high-density lipoprotein cholesterol, and triglycerides using standard enzymatic methods. Plasma HIV RNA was measured using the Cobas TaqMan HIV-1 assay (Roche Diagnostics Systems, Branchburg, New Jersey). Cryopreserved plasma was assessed by immunoassay in duplicate for plasma levels of the inflammatory markers hs-CRP (Labor Diagnostika, Nordhorn, Germany), sCD163 (Quantikine, R&D Systems, Minneapolis, Minnesota), and the bacterial translocation markers sCD14 (Quantikine, R&D Systems) and LTA (Abbexa, Cambridge, United Kingdom), according to the manufacturers' recommendations. Luminex was used to measure the concentrations

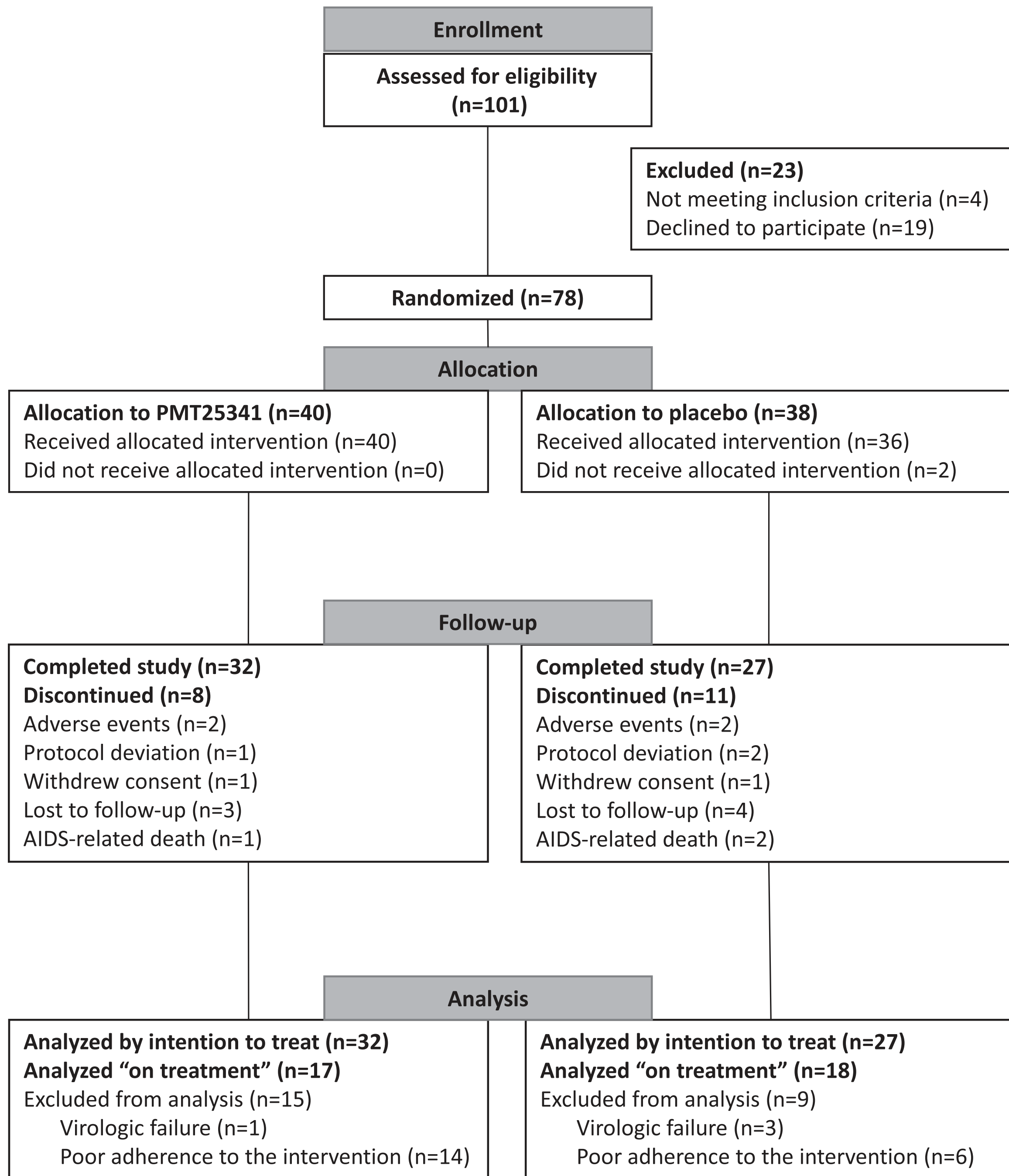

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram.

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

of 6 cytokines: IL-6, IL-7, IL-10, IL-17á, IP-10, and TNF-á (Luminex, R&D Systems), according to the manufacturers' instructions.

Markers of Adaptive Immune Activation

For T-cell immunophenotyping, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque gradient centrifugation and immediately stored in liquid nitrogen. T-cell immunophenotyping from thawed PBMCs was performed with the following antibody combination: CD3-VioBlue, CD4–fluorescein isothiocyanate, CD8-VioGreen, CD28-phycoerythrin, CD38-APC, and HLA-DR–APC-Vio770. Antibodies were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany), and isotype controls were carried out. In brief, PBMCs were incubated with the antibodies for 20 minutes at 4°C, washed, and resuspended in phosphate-buffered saline containing 1% azide. Cells were analyzed using a Gallios flow cytometer (Beckman-Coulter, Brea, California). After initially gating lymphocytes according to morphological parameters, at least 30 000 $CD3^+$ T cells were collected for each sample and analyzed with Kaluza software (Beckman-Coulter).

Statistical Methods

To estimate the required sample size, a power calculation was performed based on published $CD4^+$ T-cell count longitudinal data from a meta-analysis indicating a mean increase of $CD4^+$ T cells after 48 weeks of ART of 160 cells/µL [27]. Combined with an anticipated dropout rate of 20%, the ability to detect a 20% difference in the mean $CD4^+$ T-cell increase at week 48, with 80% power and an α error of <.05, the sample size estimation was 60 subjects.

Qualitative variables were reported as a frequency distribution whereas quantitative variables were described as median with interquartile range (IQR). Cross-sectional pairwise comparisons between groups were performed using the χ^2 test for categorical variables. Since the distribution of all the assessed variables departed from normality after Shapiro-Wilk test, we used the Mann-Whitney *U* test for the between-group comparisons of continuous variables and Wilcoxon signed-rank matched-pairs test to evaluate differences in numerical outcomes between time-points. In addition, for the primary outcome of changes in $CD4^+$ T cells, $CD8^+$ T cells, and CD4/CD8 ratio, we used linear mixed models with a random effect for each patient to allow for correlations caused by repeated observations. A robust variance estimator was used given the deviations from normality. Continuous outcome variables were log-transformed to satisfy model assumptions. Statistical analysis was performed using Stata version 15.0 (StataCorp LP, College Station, Texas). Figures were generated using Prism version 7.0 (GraphPad, La Jolla, California).

RESULTS

General Characteristics of the Study Population and Safety Data

Between 2013 and 2016, 101 participants were screened to participate in the study; 23 were not eligible and the remaining 78 were randomized into treatment or placebo groups. A total of 59 subjects completed the 48-week follow-up (Figure 1). Their mean age was 38 years, 91% were male, and 83% were men who have sex with men. The median $CD4^+$ T-cell counts were 225 (IQR, 117–288) cells/µL and the median CD4/CD8 ratio was 0.27 (IQR, 0.13–0.34). No statistically significant differences were observed among the groups in dietary habits (Supplementary Figure 1). All initiated triple ART. The general characteristics were well balanced between groups (Table 1). Of the participants who completed the follow-up, 14 (43.8%) in the active group and 9 (33.3%) in the placebo group reported adherence to the nutritional intervention <50% (*P* = .716). The lack of adherence was justified by bad taste in 3 subjects in the active group and 1 in the placebo group, and by nausea in 1 subject in the active group; the remaining cases did not provide a justification. No serious adverse events attributable to the intervention were reported.

Table 1. Baseline Characteristics

Characteristic	PMT25341 (n = 32)	Placebo (n = 27)
Age, y, mean (SD)	36 (8)	38 (14)
Male sex	28 (88)	26 (96)
Body mass index, kg/m², mean (SD)	23.6 (3.1)	22.7 (3.3)
Time since HIV diagnosis, mo, median (P25–P75)	3.1 (0.9–6.9)	1.9 (0.9–11.5)
Risk factor		
IDU	0 (0)	1 (3.7)
MSM	24 (75)	25 (92.6)
Heterosexual	5 (15.6)	1 (3.7)
Unknown	3 (9)	0 0
AIDS diagnosis	4 (12.5)	2 (7.4)
HIV RNA, copies/mL, median (P25–P75)	4.9 (4.5–5.4)	4.4 (4–5.2)
$CD4^+$ T-cell counts, cells/ìL, median (P25-P75)	226 (117–283)	221 (111–311)
$CD8^+$ T cell count, cells/ìL, median (P25–P75)	915 (532–1428)	966 (658–1273)
CD4/CD8 ratio	0.21 (0.13–0.34)	0.22 (0.12–0.41)
HCV coinfection	1 (3.1)	3 (11.1)
Use of antibiotic prophylaxis	5 (18.5)	9 (28.1)
First-line ART used		
INSTI-based	19 (59.4)	17 (63.0)
PI-based	5 (15.6)	4 (14.8)
NNRTI-based	8 (25.0)	6 (22.2)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ART, antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug user; INSTI, integrase strand transfer inhibitor; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; P25, 25th percentile; P75, 75th percentile; PI, protease inhibitor; SD, standard deviation.

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

Effects of the Nutritional Intervention on $CD4^+$ T-Cell Count, $CD8^+$ T-Cell Count, and CD4/CD8 Ratio Recovery

We sought to compare the extent of $CD4^+$ and $CD8^+$ T-cell count and CD4/CD8 ratio recovery between the treatment groups. Both $CD4^+$ T-cell counts and the CD4/CD8 ratio clearly and significantly increased with ART treatment in all groups over the 48 weeks ($P < .0001$), but there were no significant differences detected between groups (intent-to-treat and on-treatment analysis). $CD8^+$ T-cell counts remained stable over the 48-week follow-up, and no significant differences between groups were detected (Figure 2 and Table 2).

Changes in T-Cell Activation, T-Cell Senescence, Inflammation, and Bacterial Translocation Parameters

We analyzed the differences in markers of T-cell activation and inflammation after 48 weeks. Whereas the percentage of $CD4^+HLA\text{-}DR^+CD38^+$ $CD8^+HLA\text{-}DR^+CD38^+$ and $CD8^+CD28^-$ T cells significantly decreased through the 48 weeks in the overall population (P values of < .0001, <.0001, and .01, respectively) (Supplementary Table 2), no significant differences were detected between treatment groups (Table 3). Similarly, although we observed significant decreases through the 48 weeks in the grouped analyses in IL-6, sCD163, sCD14, and IP-10 (all P values <.0001) in the overall population, no consistent effects attributable to the nutritional intervention were detected.

Microbial Composition Analysis

After 48 weeks of ART, subjects did not show significant changes in α-diversity markers, including the number of species, in the Shannon, Pielou indexes, Chao1, or abundance-based coverage (ACE) estimators (Figure 3 and Table 4) or in the relative abundance of the most abundant genera in the overall population (Figure 4A). Nonmetric multidimensional scaling analysis of the composition distribution at OTU level based on the weighted UniFrac distance matrix did not reveal significant clustering of the subject's microbiota related to the study group (Figure 4B). However, linear discriminant effect size (LEfSe) analysis did indicate that those in the PMT25341 group showed an enrichment of unclassified bacteria from the Lachnospiraceae and Victivallaceae families and depletion of *Blautia* species compared with placebo (Figure 4C).

DISCUSSION

We show that among HIV-infected adults diagnosed at advanced stages of the disease, $CD4^+$ T-cell counts and CD4/CD8 ratios improved after 48 weeks of ART, with a reduction in the

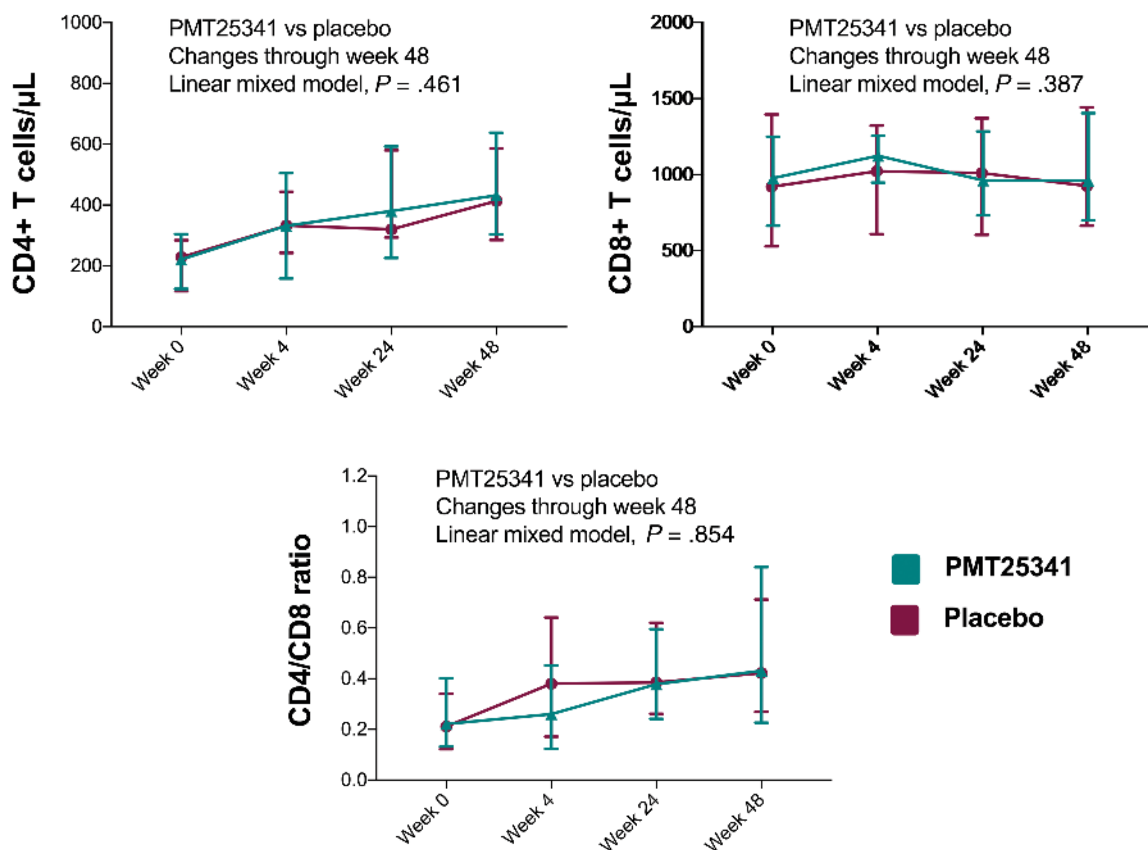

Figure 2. Effects of PMT25341 on $CD4^+$ T-cell counts, $CD8^+$ T-cell counts, and CD4/CD8 ratio (intent-to-treat population).

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

Table 2. Linear Mixed Models for Differences in CD4⁺ T Cells, CD8⁺ T Cells, and CD4/CD8 Ratio Trajectories Between Treatment Arms

Model	Coefficient	Standard Error	*P* Value
$CD4^+$ T cells			
Intercept	2.28	0.07	<.0001
Duration (wk)	0.006	0.0009	<.0001
Treatment (PMT25341)	0.0009	0.093	.939
Interaction between treatment and duration	0.008	0.001	.474
$CD8^+$ T cells			
Intercept	2.94	0.038	<.0001
Duration (wk)	−0.0003	0.0008	.975
Treatment (PMT25341)	0.017	0.050	.725
Interaction between treatment and duration	0.001	0.001	.387
CD4/CD8 ratio			
Intercept	−0.660	0.060	<.0001
Duration (wk)	0.005	0.0007	<.0001
Treatment (PMT25341)	−0.009	0.088	.913
Interaction between treatment and duration	−0.0003	0.001	.854

level of inflammatory markers, bacterial translocation, and T-cell activation. However, supplementation with PMT25341, a nutritional product specifically designed to target the different GI defects associated with HIV immunopathogenesis, did not enhance this immune reconstitution.

Strategies targeting the GALT and the microbiota in HIV-infected models have yielded inconsistent results. After preliminary observations in cohort studies suggesting a benefit of probiotics on $CD4^+$ T-cell dynamics in Africa [28], 2 controlled studies in ART-naive patients failed to demonstrate this benefit [29, 30]. Thereafter, the effects of probiotics on other outcomes has been evaluated in pilot studies in ART-treated individuals. For example, daily probiotics for 8 weeks led to a significant decrease in D-dimers, but not-CRP or IL-6 in 24 HIV-infected patients on ART [31]. A further study showed that supplementing ART-treated HIV-infected individuals with the probiotic *Saccharomyces boulardii* led to a slight decrease of a bacterial translocation marker but not other inflammatory markers [20]. Prebiotics are metabolized by gut microorganisms and the subsequent modulation of microbiota composition and/or activity is assumed to be beneficial [32]. Their use has been evaluated in HIV infection, with a significant decline of the immune activation marker sCD14 and in $CD4^+CD25^+$ T cells observed in ART-naive individuals [14]. As with our current study, others have tried to combine prebiotics, probiotics, and other compounds in an attempt to target the multiple immunological deficits in the gut. For example, Cahn et al showed that a supplement containing prebiotics, omega-3 and -6, bovine colostrum, and cysteine was associated with a slower $CD4^+$ T-cell decline in ART-naive, HIV-infected individuals [15]. We have also noted, using a combination of several prebiotics and glutamine in a pilot-controlled study, a potential beneficial effect on T-cell activation, particularly in ART-naive individuals [19].

PMT25341 included components previously associated with the enhancement of gut epithelial barrier integrity and decreased bacterial translocation. The components were as follows: prebiotics [14]; the probiotic yeast *S. boulardii* [20, 33]; the essential amino acids glutamine and arginine [34, 35]; a mixture of long-chain fatty acids with known anti-inflammatory properties [36]; vitamin D, as a deficiency predicts impaired CD4 recovery

Table 3. Comparison of Changes in T-Cell Activation, T-Cell Senescence, Inflammatory, and Bacterial Translocation Markers Between Treatment Arms

	Fold Change Through Week 48		*P* Value	
Parameter	PMT25341	Placebo	ITT	OT
T-cell activation and senescence	(n = 14)	(n = 14)		
%$CD4^+$HLA-DR^+CD38^+ T cells	0.28 (0.31–0.37)	0.29 (0.21–0.37)	.818	.591
%$CD8^+$HLA-DR^+CD38^+ T cells	0.40 (0.24–0.61)	0.33 (0.24–0.50)	.645	.879
%$CD4^+CD28^-$ T cells	1.27 (0.77–1.82)	0.70 (0.38–1.30)	.168	.706
%$CD8^+CD28^-$ T cells	0.97 (0.92–1.01)	0.95 (0.89–0.99)	.358	.173
Soluble biomarkers	(n = 30)	(n = 26)		
IL-6, pg/mL	1 (0.50–1.10)	1 (0.25–1.41)	.716	.901
hs-CRP, ng/mL	0.75 (0.37–1.29)	0.72 (0.25–1.50)	.597	.549
TNF-α, pg/mL	0.97 (0.75–4.27)	1 (0.92–4.18)	.577	.224
sCD14, ng/mL	0.85 (0.78–0.93)	0.86 (0.76–0.95)	1	.680
sCD163, ng/mL	0.58 (0.48–0.68)	0.59 (0.47–0.70)	.584	.199
IP-10, pg/mL	0.56 (0.38–0.92)	0.37 (0.24–0.52)	.036	.303
LTA, ng/mL	0.52 (0–1.36)	0.52 (0.34–1.27)	.864	.155
IL-7, pg/mL	0.45 (0.20–1.11)	0.98 (0.30–2.03)	.172	.328
IL-10, pg/mL	0.59 (0.31–1.11)	0.44 (0.26–0.65)	.338	.898
IL-17, pg/mL	0.83 (0.06–1.09)	1.12 (0.83–1.79)	.112	.261

Data are presented as XXX (XXX) unless otherwise indicated.

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; IP-10, interferon-gamma-inducible protein 10; ITT, intent-to-treat analysis; LTA, lipoteichoic acid; OT, on-treatment analysis; sCD14, soluble CD14; sCD163, soluble CD163; TNF-α, tumor necrosis factor alpha.

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

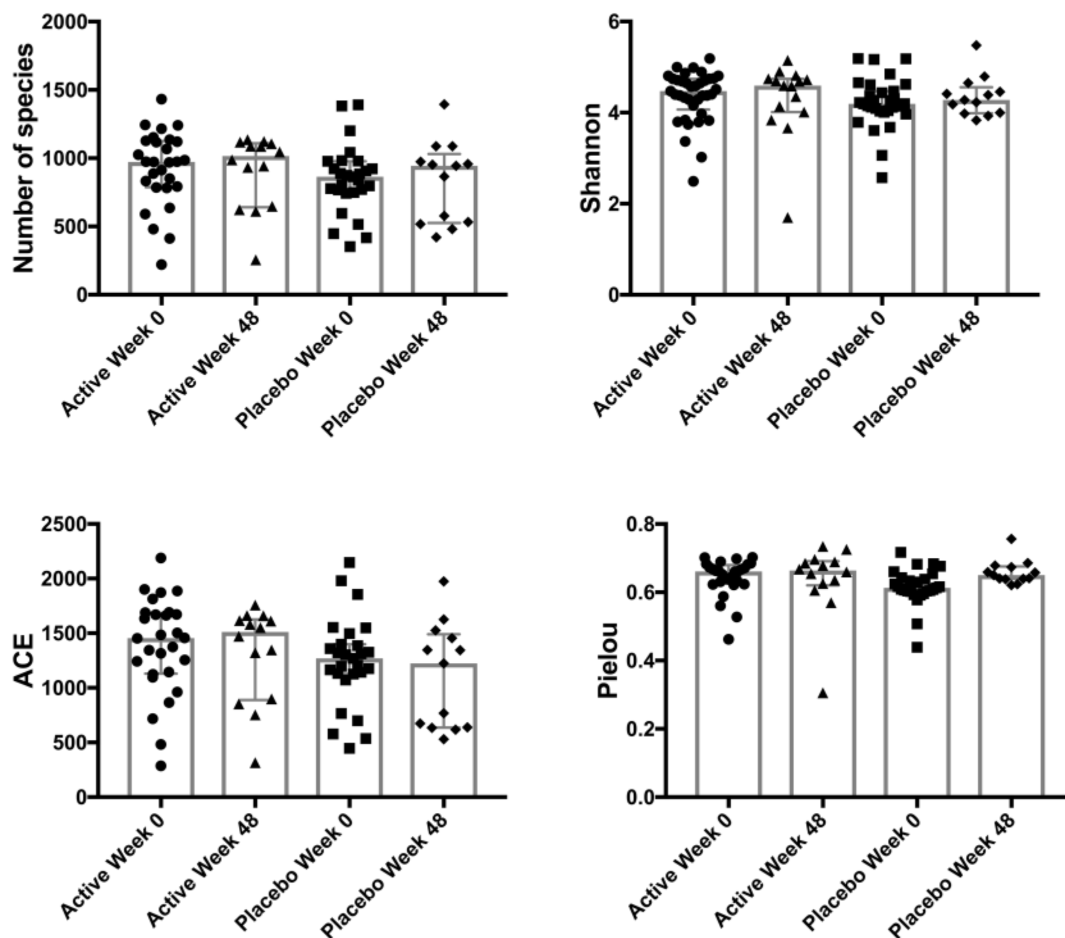

Figure 3. Alpha-diversity of taxonomic data from patients with advanced human immunodeficiency virus disease at baseline and after 48 weeks of antiretroviral therapy and PMT25341 supplementation or placebo. The α-diversity metrics represent the relative abundance and evenness of bacterial species in a community. Higher values indicate communities with greater diversity of species. We did not detect within-group differences in α-diversity metrics. Data are represented as median (P25–P75). Abbreviation: ACE, abundance-based coverage.

[37]; and AM3, an immunomodulatory glycopeptide produced by *Ricinus communis*, which induces the antiviral response of mononuclear cells and attenuates lipopolysaccharide-induced inflammation [38]. Of note, we used a high-dose combination of these compounds, which was administered daily over the 48 weeks in the late-presenting subjects, who could potentially benefit most from novel strategies boosting immune reconstitution. In contrast with some previous reports in recently diagnosed ART-naive or stable triple-ART patients [14, 15, 19, 20, 31] in whom nutritional interventions have shown a modest impact on some immunological outcomes, we did not detect any beneficial effect on these outcomes in late presenters. This could suggest that the long-term immunological consequences of a low nadir CD4^{+} cell count could overshadow the success of immunomodulatory interventions, or that the effects of ART on immune restoration are so large that they overshadow any smaller effects that might be observed with immunonutrition. However, the different nature of the interventions assessed so far in HIV-infected individuals (eg, different prebiotics, different probiotic strains) and the differences in the study populations (eg, ART-naive or ART-treated individuals) might also explain the divergent results across studies.

Table 4. Changes in α-Diversity Metrics of Fecal Microbiota, According to Study Group

	Fold Change Through Week 48		*P* Value	
Parameter	PMT25341	Placebo	ITT	OT
No. of species	0.87 (0.77–1.10)	0.79 (0.60–1.09)	.593	.795
Shannon	0.98 (0.87–1.05)	0.99 (0.96–1.03)	.662	.449
Pielou	1 (0.92–1.03)	1.05 (1.01–1.07)	.19	.131
Chao1	0.75 (0.62–0.99)	0.69 (0.48–0.96)	.56	.545
ACE	0.83 (0.72–1.05)	0.72 (0.51–1.02)	.497	.597

Data are presented as median (P50–P75).

Abbreviations: ACE, abundance-based coverage; ITT, intent-to-treat analysis; OT, on-treatment analysis.

In this study, our supplementation did not have a clear impact on gut microbiota composition, and only a few taxa from the Lachnospiraceae and Victivallaceae families were significantly

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

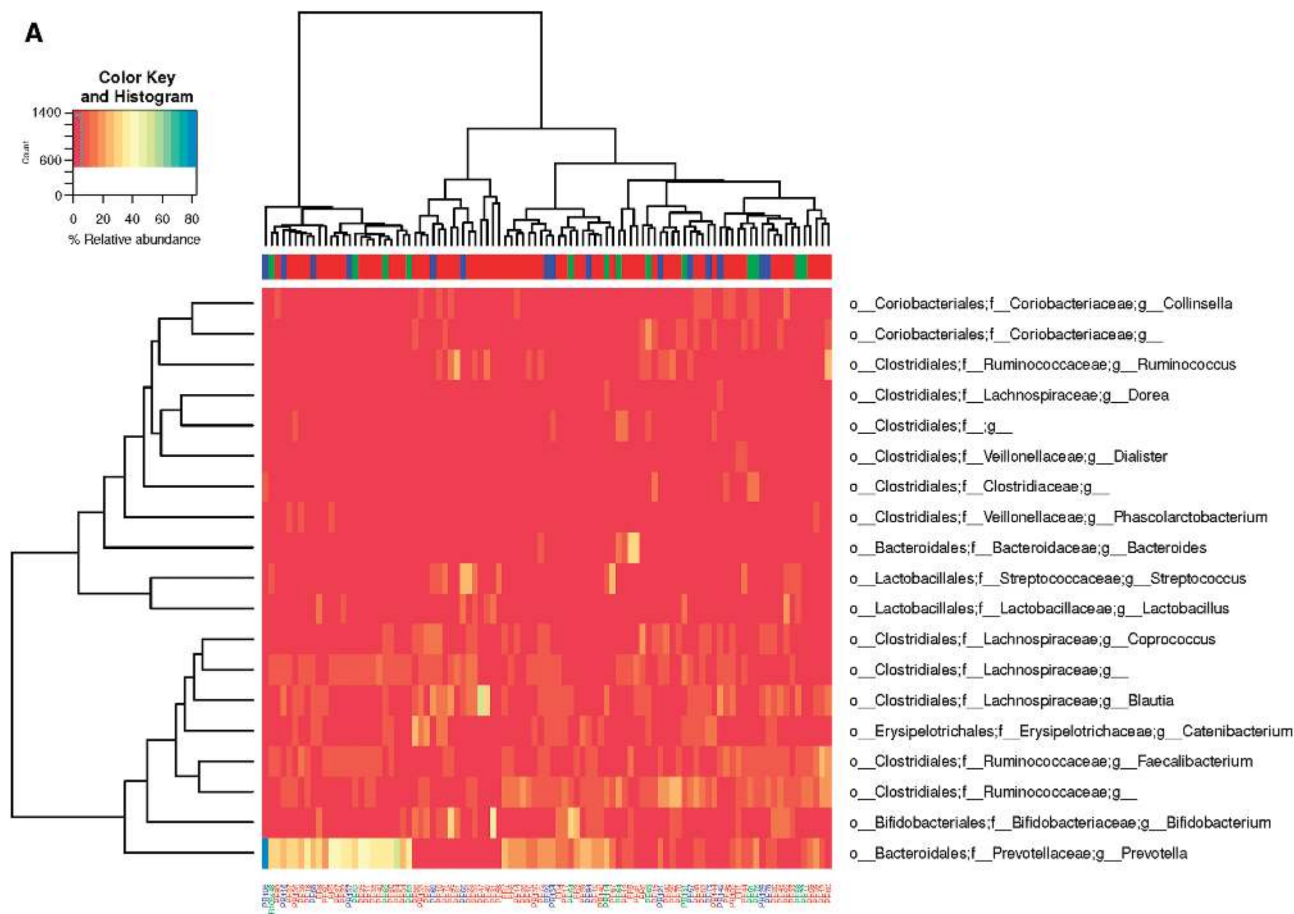

Figure 4. Beta-diversity analysis. *A*, Heatmap of the abundance of most abundant genera in the study population. Each row represents an individual genus; relative values are denoted by color below each subject column. In the first row, blue represents the abundance of these genera at baseline in the PMT25341 and placebo groups. Green represents the placebo group at week 48, and blue represents the PMT25341 group. Error bars represent the median, 25th percentile, and 75th percentile values. *B*, Nonmetric multidimensional scaling (NMDS) analysis of the composition distribution at operational taxonomic unit level based on the weighted UniFrac distance matrix. NMDS analysis is a robust method to visualize similarities or dissimilarities in high-dimensional data. In this case, it assigns each patient's microbial composition to a location in a 2-dimensional graph where the distance between any 2 samples is a measure of their similarity (smaller distance for higher similarity). The microbial composition of patients at baseline (red circles) was not significantly different from patients at week 48 treated with PMT25341 (blue) or with placebo (green). *C*, Linear discriminant *analysis* (LDA) effect size (LEfSe) of taxonomic data from patients with advanced human immunodeficiency virus disease at baseline and after 48 weeks of antiretroviral therapy plus PMT25341 supplementation or placebo. LDA is a tool to identify biomarkers from high-dimensional data of 2 or more groups using, in this case, relative abundances of bacterial taxa. This approach revealed that after 48 weeks, subjects treated with PMT25341 were significantly enriched with unclassified bacteria from the Lachnospiraceae and Victivallaceae families, but depleted in *Blautia* species.

affected by the intervention. Species from the Lachnospiraceae family are considered beneficial as they produce butyrate from the digestion of dietary fiber [39] and have previously been shown to be depleted in HIV-infected subjects [9, 40, 41]. However, despite its depletion at the compositional level, we have previously shown that the Lachnospiraceae family is among those which become most transcriptionally active during HIV infection [11], and that increases in their abundance with prebiotics correlated with decreases in inflammatory markers [19]. Only 1 study has examined the Victivallaceae family in HIV-infected subjects, where its depletion was observed [42]. So, although PMT25341 exerted only minor effects on the microbiota structure, this could be beneficial in the long term, although given the absence of any major impact here the gut microbiota of ART-treated subjects appears more resilient than that of ART-naive subjects, as previously noted [11, 19].

As main strengths, our study was double blind, randomized, placebo controlled and assessed an intervention over 48 weeks, which should have allowed for the detection of small effect sizes, and included a comprehensive analysis with an assessment of compositional changes in the microbiome. However, there were some limitations: First, a considerable number of subjects (n = 19) discontinued the study, which can be explained by the difficulties of evaluating a 48-week intervention in a population prone to experiencing adverse outcomes [43]; second, the adherence to the intervention was poorer in the active group, which was due to a fishy flavor produced by the omega-3 and -6 fatty acids of the active treatment. Finally, it is unknown whether any of the

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

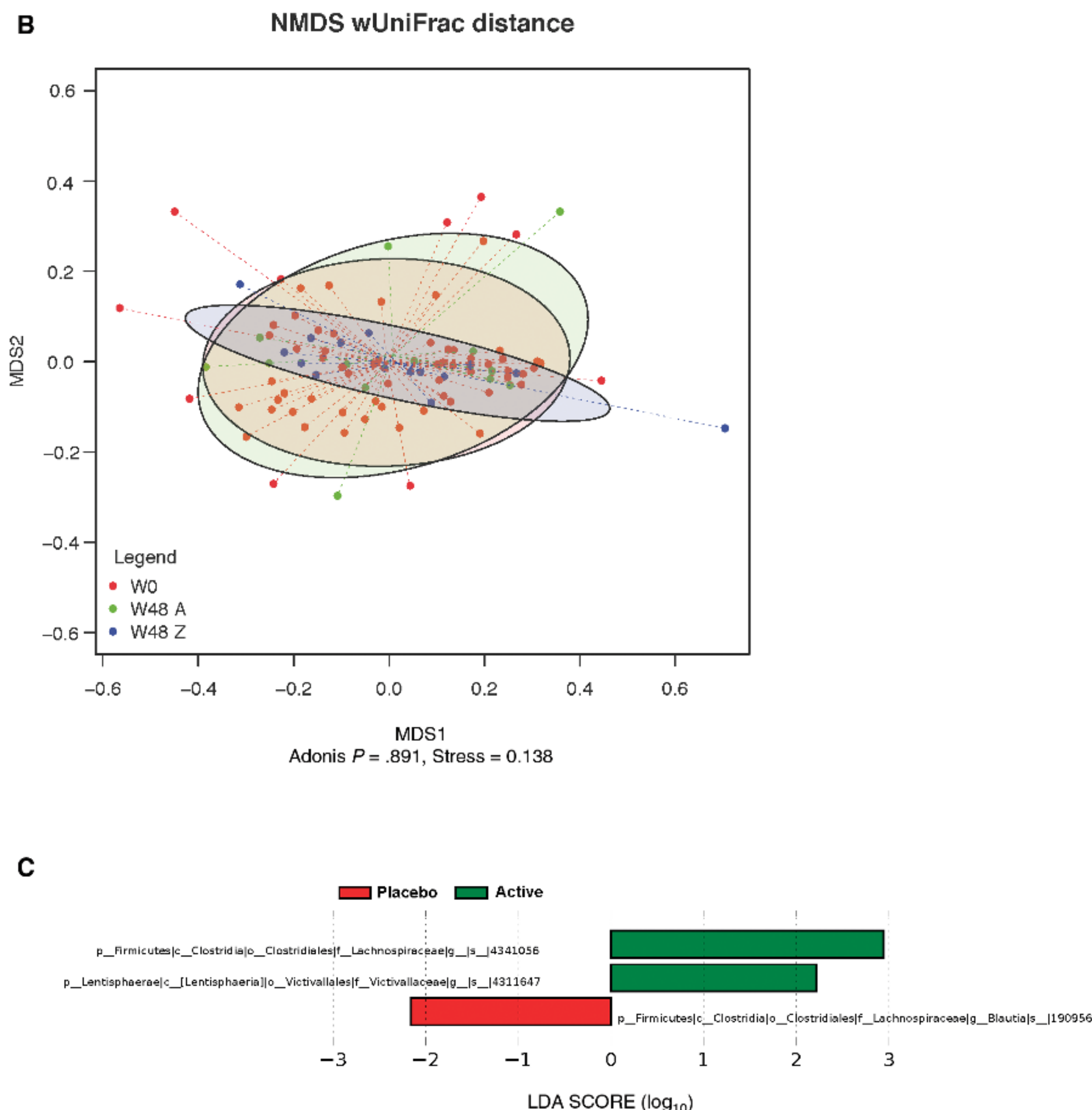

Figure 4. (Continued)

individual ingredients of PMT25341 may have proved efficacious if administered at higher doses than in the tested formulation.

In conclusion, in HIV-infected patients with a CD4 count of <350 cells/μL at diagnosis, a 48-week supplementation with a combination of compounds previously shown to exert immunomodulatory effects in HIV did not improve circulating T-cell numbers, inflammation, or immunoactivation or affected gut microbiota structure. In HIV-infected patients initiating ART at advanced disease, the clear immunological benefits of ART were not enhanced by this nutritional intervention targeting the GALT and microbiota.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank all the study participants who contributed to this work, as well as the clinical research staff of the participating institutions who made this research possible.

Disclaimer. The funding bodies did not have a role in the design or conduct of the study, the analysis and interpretation of the results, the writing of the report, or the decision to submit the manuscript for publication.

Financial support. This work was supported by the Instituto de Salud Carlos III (Plan Estatal de I+D+i 2013–2016, projects PI13/00438, AC15/00022, and PI15/00345, and the Spanish AIDS Research Network RD16/0025/0001project), the Spanish Ministry of Science and Competitiveness (project SAF2015-65878-R), and co-financed by the European Development Regional Fund "A Way to Achieve Europe." The present investigation was also funded by the Instituto de Salud Carlos III

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

and the Fundación Asociación Española contra el Cáncer within the ERA (European Research Area)-NET aligning national/regional translational cancer research programmes and activities (TRANSCAN)-2 program (grant number AC17/00022). S. S.-V. was supported by a grant from the Spanish Ministry of Science and Competitiveness (Contratos Juan Rodés, ECC/1051/2013). Nutricion Médica, S.L. manufactured and packaged the nutritional product under investigation.

Potential conflicts of interest. J. R. B. has received fees for consulting work from AbbVie, Bristol-Myers Squibb (BMS), Gilead Sciences, Janssen, Merck, and ViiV Healthcare; compensation for lectures from AbbVie, BMS, Gilead Sciences, Janssen, Merck, and ViiV; payments for the development of educational presentations for Gilead Sciences and BMS; and grants from Gilead Sciences, BMS, Merck, and ViiV. O. B. has received personal fees and nonfinancial support from Janssen, Gilead, and MSD; personal fees from AbbVie; and grants and personal fees from ViiV. J. I. B. has received grants from Gilead Sciences and personal fees from Gilead, ViiV, MSD, and Janssen. V. E. has received personal fees from Janssen, Gilead, and ViiV and grants from MSD and Gilead. M. d. L. has received honoraria (grants and personal fees) as a speaker in educational programs sponsored by Janssen, ViiV, and Gilead; and has received support (registration, travel assistance) for expert courses and congresses by MSD and ViiV. M. M. has received personal fees from BMS and MSD. A. C. has received personal fees from ViiV, MSD, and Gilead. S. S.-V. has received grants from MSD and Gilead, and personal fees from ViiV, MSD, Gilead, and Janssen. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Mocroft A, Lundgren JD, Sabin ML, et al. Risk factors and outcomes for late presentation for HIV-positive persons in Europe: results from the Collaboration of Observational HIV Epidemiological Research Europe Study (COHERE). PLoS Med **2013**; 10:e1001510.
2. Sobrino-Vegas P, Moreno S, Rubio R, et al. Impact of late presentation of HIV infection on short-, mid- and long-term mortality and causes of death in a multicenter national cohort: 2004–2013. J Infect **2016**; 72:587–96.
3. Antinori A, Coenen T, Costagiola D, et al; European Late Presenter Consensus Working Group. Late presentation of HIV infection: a consensus definition. HIV Med **2011**; 12:61–4.
4. Trickey A, May MT, Vehreschild JJ, et al. Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies. Lancet HIV **2017**; 4:e349–56.
5. Gandhi RT, McMahon DK, Bosch RJ, et al. ACTG A5321 Team. Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation. PLoS Pathog **2017**; 13:e1006285.
6. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med **2006**; 12:1365–71.
7. Mavigner M, Cazabat M, Dubois M, et al. Altered CD4$^+$ T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. J Clin Invest **2012**; 122:62–9.
8. Sandler NG, Douek DC. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. Nat Rev Microbiol **2012**; 10:655–66.
9. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med **2013**; 5:193ra91.
10. Vázquez-Castellanos JF, Serrano-Villar S, Latorre A, et al. Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals. Mucosal Immunol **2015**; 8:760–72.
11. Serrano-Villar S, Rojo D, Martínez-Martínez M, et al. Gut bacteria metabolism impacts immune recovery in HIV-infected individuals. EBioMedicine **2016**; 8:203–16.
12. Somsouk M, Estes JD, Deleage C, et al. Gut epithelial barrier and systemic inflammation during chronic HIV infection. AIDS **2015**; 29:43–51.
13. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis **2014**; 210:1248–59.
14. Gori A, Rizzardini G, Van't Land B, et al. Specific prebiotics modulate gut microbiota and immune activation in HAART-naive HIV-infected adults: results of the 'COPA' pilot randomized trial. Mucosal Immunol **2011**; 4:1–10.
15. Cahn P, Ruxrungtham K, Gazzard B, et al; (BTE) Blinded Nutritional Study for Immunity and Tolerance Evaluation Study Team. The immunomodulatory nutritional intervention NR100157 reduced CD4$^+$ T-cell decline and immune activation: a 1-year multicenter randomized controlled double-blind trial in HIV-infected persons not receiving antiretroviral therapy (the BITE Study). Clin Infect Dis **2013**; 57:139–46.
16. Asmuth DM, Ma ZM, Albanese A, et al. Oral serum-derived bovine immunoglobulin improves duodenal immune reconstitution and absorption function in patients with HIV enteropathy. AIDS **2013**; 27:2207–17.
17. Somsouk M, Dunham RM, Cohen M, et al. The immunologic effects of mesalamine in treated HIV-infected individuals with incomplete CD4$^+$ T cell recovery: a randomized crossover trial. PLoS One **2014**; 9:e116306.
18. Villar-García J, Güerri-Fernández R, Moya A, et al. Impact of probiotic *Saccharomyces boulardii* on the gut microbiome composition in HIV-treated patients: a double-blind, randomised, placebo-controlled trial. PLoS One **2017**; 12:e0173802.
19. Serrano-Villar S, Vázquez-Castellanos JF, Vallejo A, et al. The effects of prebiotics on microbial dysbiosis, butyrate production and immunity in HIV-infected subjects. Mucosal Immunol **2017**; 10:1279–93.
20. Villar-García J, Hernández JJ, Güerri-Fernández R, et al. Effect of probiotics (*Saccharomyces boulardii*) on microbial translocation and inflammation in HIV-treated patients: a double-blind, randomized, placebo-controlled trial. J Acquir Immune Defic Syndr **2015**; 68:256–63.
21. Grupo de estudio del SIDA/Plan nacional de salud (GESIDA/PNS). Guidelines for the use of antiretroviral agents in HIV-infected adults, **2016**. Available at: https://www.msssi.gob.es/ciudadanos/enfLesiones/enfTransmisibles/sida/publicaciones/profSanitarios/docTARGesidaPNS2013Def.pdf. Accessed 19 September 2016.
22. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods **2010**; 7:335–6.
23. Cole JR, Wang Q, Cardenas E, et al. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res **2009**; 37:D141–5.
24. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics **2010**; 26:266–7.
25. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics **2010**; 26:2460–1.
26. Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One **2010**; 5:e9490.
27. Bartlett JA, DeMasi R, Quinn J, Moxham C, Rousseau F. Overview of the effectiveness of triple combination therapy in antiretroviral-naive HIV-1 infected adults. AIDS **2001**; 15:1369–77.
28. Irvine SL, Hummelen R, Hekmat S, Looman CW, Habbema JD, Reid G. Probiotic yogurt consumption is associated with an increase of CD4 count among people living with HIV/AIDS. J Clin Gastroenterol **2010**; 44:e201–5.
29. Hummelen R, Hemsworth J, Changalucha J, et al. Effect of micronutrient and probiotic fortified yogurt on immune-function of anti-retroviral therapy naive HIV patients. Nutrients **2011**; 3:897–909.
30. Hummelen R, Changalucha J, Butamanya NL, et al. Effect of 25 weeks probiotic supplementation on immune function of HIV patients. Gut Microbes **2011**; 2:80–5.
31. Stiksrud B, Nowak P, Nwosu FC, et al. Reduced levels of D-dimer and changes in gut microbiota composition after probiotic intervention in HIV-infected individuals on stable ART. J Acquir Immune Defic Syndr **2015**; 70:329–37.
32. Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol **2015**; 12:303–10.
33. Generoso SV, Viana ML, Santos RG, et al. Protection against increased intestinal permeability and bacterial translocation induced by intestinal obstruction in mice treated with viable and heat-killed *Saccharomyces boulardii*. Eur J Nutr **2011**; 50:261–9.
34. De-Souza DA, Greene LJ. Intestinal permeability and systemic infections in critically ill patients: effect of glutamine. Crit Care Med **2005**; 33:1125–35.
35. Viana ML, Santos RG, Generoso SV, Arantes RM, Correia MI, Cardoso VN. Pretreatment with arginine preserves intestinal barrier integrity and reduces bacterial translocation in mice. Nutrition **2010**; 26:218–23.
36. Barham JB, Edens MB, Fonteh AN, Johnson MM, Easter L, Chilton FH. Addition of eicosapentaenoic acid to gamma-linolenic acid-supplemented diets prevents serum arachidonic acid accumulation in humans. J Nutr **2000**; 130:1925–31.

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022

37. Aziz M, Livak B, Burke-Miller J, et al. Vitamin D insufficiency may impair CD4 recovery among Women's Interagency HIV Study participants with advanced disease on HAART. AIDS **2013**; 27:573–8.
38. Majano P, Alonso-Lebrero JL, Janczyk A, et al. AM3 inhibits LPS-induced iNOS expression in mice. Int Immunopharmacol **2005**; 5:1165–70.
39. Vital M, Karch A, Pieper DH. Colonic butyrate-producing communities in humans: an overview using omics data. mSystems **2017**; 2. doi:10.1128/mSystems.00130-17.
40. Mutlu EA, Keshavarzian A, Losurdo J, et al. A compositional look at the human gastrointestinal microbiome and immune activation parameters in HIV infected subjects. PLoS Pathog **2014**; 10:e1003829.
41. Ling Z, Jin C, Xie T, Cheng Y, Li L, Wu N. Alterations in the fecal microbiota of patients with HIV-1 infection: an observational study in a Chinese population. Sci Rep **2016**; 6:30673.
42. Rocafort M, Noguera-Julian M, Guillén Y, et al. The gut microbiota correlates with HIV control and immune status. Abstract 261. In: Conference on Retroviruses and Opportunistic Infections, Seattle, WA, 23–26 February 2015.
43. Lundgren JD, Babiker AG, Gordin F, et al; INSIGHT START Study Group. Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med **2015**; 373:795–807.

Downloaded from https://academic.oup.com/cid/article/68/1/120/4996926 by U.S. Department of Justice user on 16 August 2022